Commentary

DNA structure from A to B

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P. Shing Ho and his colleagues at Oregon State and Berkeley publish in this issue of PNAS an interesting study (1) of helical structure in the DNA hexamer GGCGCC, finding that various states that appear to be logical intermediates between A-DNA and B-DNA can be induced by methylation or bromination of cytosine or by crystal packing. Their results bear on three issues that have been argued over in the past: (*i*) the differences between A-DNA and B-DNA and transitions between them, (*ii*) the intrinsic sequence-dependent malleability of a DNA duplex, and (*iii*) the effects of local helix packing on DNA fine-structure.

The six x-ray crystal structure analyses that provided the proposed intermediates in this and an earlier study (2) are listed in Table 1. The first three are straightforward, with one DNA helix per asymmetric unit or eight helices per tetragonal unit cell. The next three, however, are unusual. These crystals have three or four distinct hexamer helices per asymmetric unit, identical in sequence and crystallizing conditions, but differing in their local environment within the resulting crystals. Vargason *et al.* (1, 2) interpret these helical structures as intermediates between true A and B structures, characterizing them as composite (helices e and f), extended (helices g and h), or A/B intermediates (helices i, j, and k). Helices a–d are the B form, and helices l and m are the A form. Together these are stated to form a series of 13 hexamers (see their figure 2 in ref. 1) that bridges the entire range from B-DNA to A-DNA. (The reuse of label i in their table 2 in ref. 1 would appear to be a nomenclature error; if so, their structural progression then has 14 members rather than 13.)

All three unique helices in the tetragonal crystals of GGCGGG + spermine adopt the B form. But the four independent molecules in each of the trigonal crystal forms having methylated or brominated C-3 exhibit at least three different helical types, which leaves the reader puzzled and wishing to know more. Three of the four helices in one crystal are said to form "a planar canopy in the a-b plane," whereas the fourth helix rises perpendicular to this canopy along the c axis. Of the three helices in the canopy, one is A-DNA whereas the other two are A/B intermediates similar to that reported earlier for CATGGGCCCATG (3) and proposed for CCCCGGGG and GGGGCCCC in solution (4, 5). This perturbation of DNA helix structure by crystal environment cries out for careful analysis.

The resulting 13 (or 14) helical structures can be grouped into a series extending from B-DNA to A-DNA, which is attempted in figures 2 and 3 of Vargason *et al.* (1). The most compelling parameter for their sequential arrangement is x-displacement or the displacement of a base pair away from the helix axis in the direction of the base pair short axis. This feature makes B-DNA (with near-zero x-displacement) a stack of base pairs along the helix axis and gives A-DNA (with large negative x-displacement) its central hole (6, 7).

If a single parameter must be chosen with which to follow the B-to-A helix transition, then x-displacement obviously is the parameter of choice. The inclination of a base pair away from perpendicularity to the helix axis, measured along its long axis (see definitions and diagrams in refs. 8–11), is less helpful than one would expect. It has been common or received knowledge from fiber diffraction that base pairs in B-DNA were perpendicular to the helix axis, whereas those in A-DNA were inclined by 15° to 20°. Yet single crystal structure analyses have failed to bear that out; observed inclinations of base pairs in A-DNA crystals are much less, generally in the range of 5° to 12°. The present study of Vargason *et al.* (1) shows little or no correlation between helix type and base pair inclination angle, unless the composite and extended helices e–h are arbitrarily deleted from the analysis.

Part of the problem with inclination as a parameter is that, in short helical segments such as these hexamers, inclination can be confused with local helix bending. The change in base pair orientation from one end of helix d to the other is easy to see in their figure 2 (1), for example. It can be ascribed to inclination, but much the same appearance would result if the helix segment were simply bent at its center in a direction that compresses the major groove at the right. Indeed, if the hexamer helix were bent at its center, then the inclination relative to an overall unbent axis would have one sense at the beginning of the helix, the opposite sense one-half turn of helix later, and the original sense again after a full turn. Careful examination of their figure 3 shows that this reversal of inclination is exactly what is observed in helix d and to a lesser extent in helices c, e, g, and h. Hence there is no compelling evidence for changes in inclination along the B-to-A series, but only for local helix bending, the latter probably induced by crystal packing. This inclination vs. bending issue illustrates how longer runs of helix than a half-turn hexamer become critical.

The parameter slide or relative motion of two stacked base pairs along their long axes (see refs. 8–11) does appear to map the transition between B and A well. This

Table 1. Crystals of proposed A/B intermediates

Sequence	Space group	a.u. per cell	Volume per cell	Helices per a.u.	Vol. per base pair	Helix labels
GGCGCC + Co^{3+}	$P4_122$	8	114,900	1	2400	a
GGCG^{br}CC	$P4_32_12$	8	90,100	1	1870	g
GGCG^{me}CC	$P4_32_12$	8	93,700	1	1950	h
GGCGCC + sper	$P4_12_12$	8	304,700	3	2100	b, c, d
GG^{br}CGCC	$P3_221$	6	295,800	4	2050	f, i, j, l
GG^{me}CGCC	$P3_221$	6	310,200	4	2150	e, i, k, m

^{br}C, 5-bromocytosine; ^{m}C, 5-methylcytosine; sper, spermine; a.u., asymmetric units. Volumes are in Å^3.

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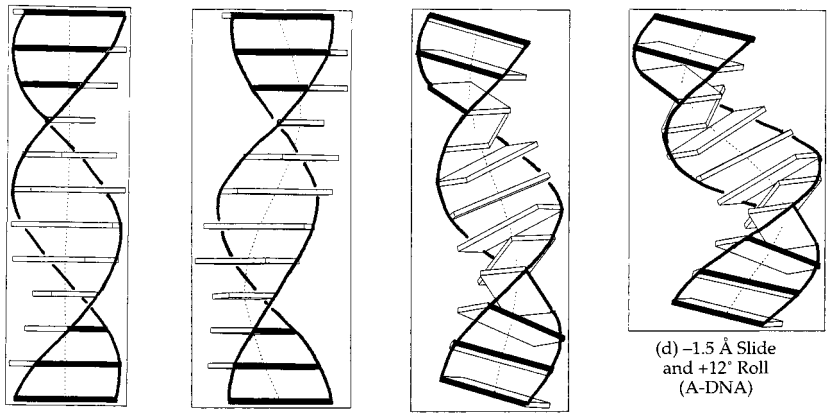

Fig. 1. Two-step conversion of B-DNA to A-DNA by varying the two helix parameters slide and roll. (*a*) Initial idealized B-DNA. (*b*) Intermediate structure obtained by application of a uniform slide of −1.5 Å from one base pair to the next. (*c*) Alternative intermediate produced by applying a uniform roll of +12°. (*d*) A-DNA reached by varying both slide and role simultaneously. [Reproduced with permission from ref. 3 (Copyright 2000, National Academy of Sciences; original proposal by Calladine and Drew (12).]

slide behavior confirms an early, and still cogent, explanation by Calladine and Drew (12) of the B-to-A transition as being driven by base stacking and involving the two helix parameters slide and roll. Fig. 1 shows the effect on an ideal B-DNA helix of first applying (*b*) a −1.5-Å slide or (*c*) a + 12° roll, and then (*d*) applying both slide and roll simultaneously. Slide without roll pushes the base pairs off-axis and creates the central hole that is typical of fiber A-DNA, and roll without slide forces the base pairs to become inclined to the helix axis without displacing them from that axis. Slide plus roll creates the familiar idealized A-DNA helix, in which base pairs are both inclined (to some degree) and shifted off the helix axis. It would be interesting to examine all of the helices of the Vargason *et al.* studies (1, 2), to see whether they could be mapped successfully onto a Calladine/Drew transition pathway.

Deoxyribose sugar pucker correlates well with slide. All of the helices with large negative slide of *ca.* −2.5 Å, g–m, exhibit the C3′-*endo* sugar conformation of A-DNA. Those helices with little or no negative slide, a–d, are described as having the C2′-*endo* sugars that conventionally are associated with B-DNA. The two intermediate, extended helices e and f, are hybrids in which the first three sugars along each strand are C3′-*endo* whereas the last three sugars vary from C2′-*endo* through C1′-*exo* to O4′-*endo* (see ideal conformation progression in Fig. 2).

There is a sound mechanical reason why high-slide helices should exhibit C3′-*endo* sugars whereas low-slide helices are more variable. As Fig. 3*A* shows, sliding base pair 1 to the right over base pair 2 pushes sugar ring 1 against the phosphate group, and the resulting pressure is relieved by allowing the 3′ carbon of the sugar ring to buckle toward the side of the ring away from the phosphate—to the *endo* side. Hence a high-slide A-DNA helix requires C3′-*endo* sugar puckering. This strain is absent in the B form (Fig. 3*B*) with essentially zero slide, and its sugar rings consequently are free to adopt a greater range of conformations, from C3′-*exo* through C2′-*endo* to C1′-*exo* to O4′-*endo* to C4′-*exo* to the C3′-*endo* that is demanded by an A helix. Fig. 2 illustrates how restricted the A-DNA helix is and how broad the distribution for B-DNA is.

In summary, what is to be concluded from the Vargason *et al.* studies of GGCGCC-based hexamers? The first caution is that one must be careful about overinterpreting the geometry of a short half-turn of helix; inclination can be confused with bending, for example. In addition, it is sobering to consider that different helix types can be observed at different locations in the same crystal form, that the DNA helix itself is highly malleable and susceptible to outside influences. Yet in a sense that is one of the strengths of DNA. It is not a rigid, repetitive helix; neither is it a rigid entity with sequence-determined local structural variations. Rather, as analyses of DNA alone (13) and in complexes with proteins (14–16) have both shown, the DNA duplex exhibits sequence-dependent deformability, or a potential for being deformed that varies from one part of the base sequence to another.

This significant demonstration by Vargason *et al.* of true intermediates between A-DNA and B-DNA, obtained under relatively mild conditions, shows that A-DNA and B-DNA are not two separate, isolated conformations.

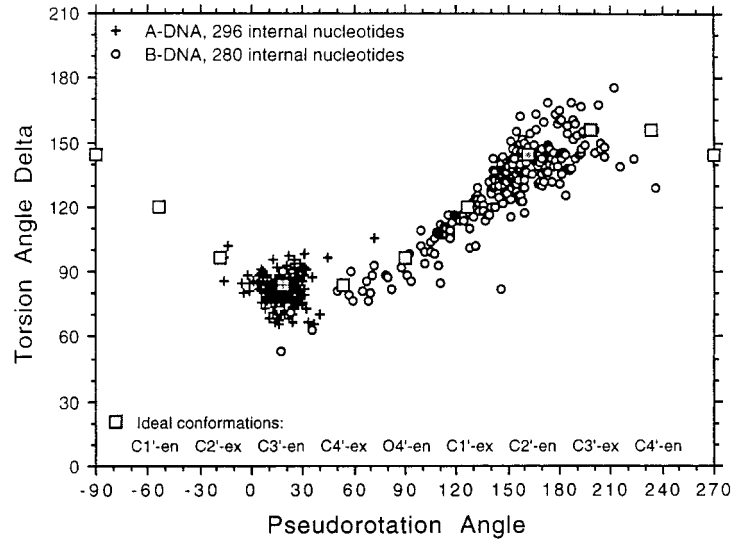

Fig. 2. Distribution of torsion angle δ vs. pseudorotation angle in sugar rings of B-DNA (○) and A-DNA (crosses), as observed in single-crystal structure analyses. Theoretical correlations between δ and pseudorotation angle are marked by □. Note that sugar conformations in A-DNA are grouped tightly around the expected C3′-*endo* conformation, whereas the B helix permits a much broader range of sugar conformations. B-DNA is not limited exclusively to the C2′-*endo* region as is often assumed. [Reproduced with permission from ref. 7 (Copyright 2001, International Union of Crystallography].

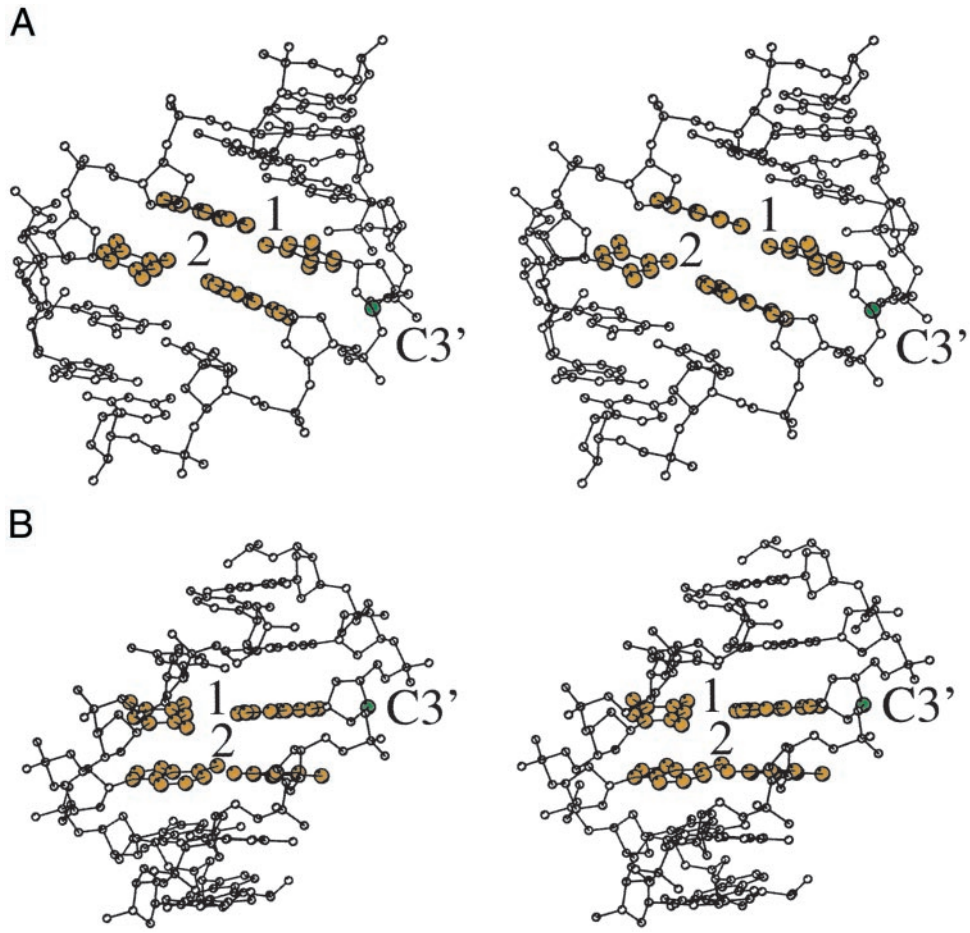

Fig. 3. (*A*) Segment of a typical A-DNA helix, of sequence GGTATACC (17). Negative slide pushes the base pair marked 1 to the right, relative to base pair 2 just below it. This move forces sugar ring 1 against phosphate group 2, and strain is relieved by allowing atom C3′ of ring 1 to move to the far side of the ring, away from the phosphate. This C3′-*endo* conformation is demanded by A-DNA. (*B*) Segment of a typical B-DNA helix, of sequence CGCGAATTCGCG (18). Now base pairs 1 and 2 have essentially zero slide between them, and no strain is felt at sugar ring 1, which is free to adopt a C2′-*endo* conformation. However, it is not restricted to C2′-*endo* and can adopt the broad range of conformations seen in Fig. 2.

Rather, they resemble the distinctions between liberal and conservative in the political arena. Everyone can define the difference between a liberal and a conservative, which is a legitimate distinction. Yet the differences offered will vary with the speaker, and an ideological continuum exists from one camp to the other. One person's conservative can be another person's liberal (especially if he is snubbed by the White House). We all can define the extreme prototypes, but woe unto whomever shall maintain that one extreme must have one set of properties and only those, whereas the other extreme must have opposite properties. A-DNA and B-DNA are useful boundary extremes with which to classify DNA, but they in no sense are bottomless local potential minima. Intermediates do exist and exist under the mildest of conditions.

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